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Vaccine immunology

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Introduction

To generate vaccine-mediated protection is a complex challenge. Currently available vaccines have largely been developed empirically, with little or no understanding on how they activate the immune system. Their early protective efficacy is primarily conferred by the induction of antigen-specific antibodies (Box 2–1). However, there is more to antibody-mediated pro-tection than the peak of vaccine-induced antibody titers. The quality of such antibody responses, e.g., their avidity, has been identified as a determining factor of efficacy. In addition, long-term protection requires the persistence of vaccine antibodies and/or the generation of immune memory cells capable of rapid and effective reactivation upon subsequent microbial exposure. The determinants of immune memory induction, as well as the relative contribution of persisting antibodies and of immune memory to protection against specific diseases, are thus essential parameters of long-term vaccine efficacy. The predominant role of B cells in the efficacy of current vaccines should not shadow the importance of T cell responses: T cells are essential to the induction of high-affinity antibodies and immune memory, and novel vaccine targets have been identified against which T cells are likely to be the prime effectors.

New methods have emerged allowing us to assess a growing number of vaccine-associated immune parameters, including in humans. This development raises new questions relative to the optimal markers to assess and their correlation with vaccine-induced protection. The identification of immune correlates—or at least surrogates—of vaccine efficacy is a major asset for the development of new vaccines or the optimization of immunization strategies using available vaccines. Thus, their determination generates a considerable amount of interest at all levels, from the immunologist working at the bench to the physician wishing to optimize a vaccine schedule for a specific patient. The tailoring of vaccine strategies for specific vulnerable populations, being the very young, the elderly or the immunosuppressed, is also largely relying on a better understanding of what supports or limits vaccine efficacy under special circumstances.

Last, the exponential development of new vaccines raises many questions that are not limited to the targeted diseases and the potential impacts of their prevention, but address the specific and non-specific impacts of such vaccines on the immune system, and thus on health in general. These immune-related concerns have largely spread into the population and questions related to the immunological safety of vaccines, i.e., to their capacity of triggering non-antigen specific responses possibly leading to conditions such as allergy, autoimmunity or even premature death are being raised. The objective of this chapter is to extract from the complex and rapidly evolving field of immunology the main concepts that are useful to better address these important questions.

How do vaccines mediate protection?

Disease control or elimination requires the induction of protective immunity in a sufficient proportion of the population. This is best achieved by immunization programs capable of inducing long-term protection, a hallmark of adaptative immunity that contrasts to the brisk but short-lasting innate immune responses. Long-term immunity is conferred by the maintenance of antigen-specific immune effectors and/or by the induction of immune memory cells that may be sufficiently efficient and rapidly reactivated into immune effectors in case of pathogen exposure.

Vaccine-induced immune effectors (Table 2–1) are essentially antibodies—produced by B lymphocytes—and capable of binding specifically to a toxin or a pathogen.[1] Other potential effectors are cytotoxic $CD8^+$ T lymphocytes* (CTL) that may limit the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines. The generation and maintenance of both B and $CD8^+$ T cell responses is supported by growth factors and signals provided by $CD4^+$ T helper (Th) lymphocytes, which are commonly subdivided into T helper 1 (Th1) and T helper 2 (Th2) subtypes* (Table 2–1). These effectors are controlled by regulatory T cells (Treg) that are involved in maintaining immune tolerance.[2] Most antigens and vaccines trigger both B and T cell responses, such that there is no rationale in opposing antibody production ('humoral immunity') and T cell responses ('cellular immunity'). In addition, $CD4^+$ T cells are required for most antibody responses, while antibodies exert significant influences on T cell responses to intracellular pathogens.[3]

Which are the main effectors of vaccine responses?

The nature of the vaccine exerts a direct influence on the type of immune effectors that are predominantly elicited and mediate protective efficacy (Table 2–2).

Capsular polysaccharides (PS) elicit B cell responses in what is classically reported as a T-independent manner*,[4,5] although increasing evidence supports a role for $CD4^+$ T cells in such

Box 2–1 Main Immunological Definitions

Adjuvant:
Agents which increase the stimulation of the immune system by enhancing antigen presentation (depot formulation, delivery systems) and/or by providing co-stimulation signals (immunomodulators). Aluminium salts are most often used in today's vaccines.

Affinity, avidity:
The antibody affinity refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen, i.e., to the strength of the interaction. The avidity is the sum of the epitope-specific affinities for a given antigen. It directly relates to its function.

Affinity maturation:
Processes through which antigen-specific B cells undergo somatic hypermutation and affinity-based selection, resulting into B cells that produce antibodies with increased affinity over germ-line antibodies.

Antibodies:
Proteins of the immunoglobulin family, present on the surface of B lymphocytes, secreted in response to stimulation, that neutralize antigens by binding specifically to their surface.

Antigen presenting cells:
Cells that capture antigens by endo- or phagocytosis, process them into small peptides, display them at their surface through MHC molecules and provide co-stimulation signals that act synergistically to activate antigen-specific T cells. Antigen presenting cells include B cells, macrophages and dendritic cells, although only dendritic cells are capable of activating naïve T cells.

B lymphocytes:
Cells that originate in the bone marrow, mature in secondary lymphoid tissues, become activated in the spleen/nodes when their surface immunoglobulins bind to an antigen and differentiate either in antibody secreting cells (plasma cells) or in memory B cells.

Carrier protein:
A protein that is used as a template to which polysaccharide moieties are chemically conjugated to generate glycoconjugate vaccines. It is currently considered that carrier proteins provide antigenic epitopes for recognition by $CD4^+$ helper T cells, in particular follicular helper T cells.

$CD4^+$ T helper 1 lymphocytes:
$CD4^+$ T cells that upon activation differentiate into cells that mainly secrete IL-2, IFN-γ and TNF-β, exerting direct antimicrobial functions (viruses) and essentially providing support to cytotoxic T cells and macrophages.

$CD4^+$ T helper 2 lymphocytes:
$CD4^+$ T cells that upon activation differentiate into cells that mainly secrete IL-4, IL-6, IL-6, IL-10, IL-13, exerting direct antimicrobial functions (parasites) and essentially providing support to B lymphocytes.

Central memory T cells:
Memory T cells trafficking through the lymph nodes, ready to proliferate and generate a high number of effector cells in response to specific microbial peptides.

Chemokines:
Small secreted proteins that function as chemoattractants, recruiting cells that express the corresponding chemokine receptors at their surface and thus migrate towards higher concentrations of chemokines.

Costimulatory molecules:
Molecules that become expressed at the surface of antigen presenting cells upon activation and deliver stimulatory signals to other cells, namely T and B cells.

Dendritic cells:
Cells that constantly sample the surroundings for pathogens such as viruses and bacteria, detect dangers and initiate immune responses. Immature patrolling DCs have a high endocytic activity and low T cell activation potential. Contact with a pathogen induces maturation and the expression of certain cell-surface molecules, greatly enhancing their ability to activate T cells.

Effector memory T cells:
Memory T cells patrolling through the body to detect specific microbial peptides and capable of an immediate cytotoxic function in case of recognition.

Extrafollicular reaction:
B cell differentiation pathways that occur outside of germinal centers, in response to protein or polysaccharide antigens. Rapid, it generates B cells that are short-lived (days) and produce low-affinity antibodies, without inducing immune memory.

Follicular dendritic cells:
Stromal cells in spleen and nodes that upon activation express chemokines (notably CXCL13) attracting activated antigen-specific B and T cells, and thus nucleate the germinal center reaction. FDCs provide anti-apoptotic signals to GC B cells and support their differentiation into plasma cells or memory B cells.

Follicular helper T lymphocytes:
CD4+ T cells that upon activation migrate towards follicular dendritic cells and provide a most critical help to germinal center B cells, influencing isotype switching, affinity maturation and differentiation.

Germinal centers:
Dynamic structure that develop in spleen/nodes in response to an antigenic stimulation and dissolves after a few weeks. GCs contain a monoclonal population of antigen-specific B cells that proliferate and differentiate through the support provided by follicular dendritic cells and helper T cells. Immunoglobulin class switch recombination, affinity maturation, B cell selection and differentiation into plasma cells or memory B cells essentially occur in GCs.

Isotype switching:
Switch of immunoglobulin expression and production from IgM to IgG, IgA or IgE, occurring during B cell differentiation through DNA recombination.

Marginal zone:
The marginal zone is the area between the red pulp and the white pulp of the spleen. Its major role is to trap particulate antigens from the circulation and present it to lymphocytes.

Regulatory T cells:
T cells that upon activation differentiate into cells that express specific cytokines (IL-10, TGF-β/surface markers) and act to suppress the activation of the immune system through various mechanisms, maintaining immune homeostasis and tolerance to self antigens.

Somatic hypermutation:
Process that introduces random mutation in the variable region of the B cell receptor (i.e., immunoglobulin) locus at an extremely high rate, during B cell proliferation. This mechanism occurs through the influence of the activation-induced cytidine deaminase (AID) enzyme and generates antibody diversification.

T lymphocytes:
Cells that originate in the thymus, mature in the periphery, become activated in the spleen/nodes if 1) their T cell receptor bind to an antigen presented by an MHC molecule and 2) they receive additional costimulation signals driving them to acquire killing (mainly $CD8^+$ T cells) or supporting (mainly $CD4^+$ T cells) functions.

T-independent B cell responses:
Differentiation pathway of B cells, mainly elicited by polysaccharides, that takes place in the marginal zone and extrafollicular areas of spleen/nodes. Its hallmarks are to be rapid (days) but to elicit the transient (months) production of antibodies of low affinity, without inducing immune memory.

T-dependent B cell responses:
Differentiation pathway of B cells elicited by protein antigens that recruits T and B cells into germinal centers of spleen/nodes. Its hallmarks are to be slow (weeks) but to elicit long-lasting (years) production of antibodies of high affinity, and immune memory.

Toll-like receptors:
Family of 10 receptors (TLR1 to TLR10) present at the surface of many immune cells, which recognize pathogens through conserved microbial patterns and activate innate immunity when detecting danger.

Table 2–1 Effector Mechanisms Triggered by Vaccines

- Antibodies prevent or reduce infections by extra- and intracellular agents and clear extracellular pathogens through :
  - binding to the enzymatic active sites of toxins or preventing their diffusion
  - neutralizing viral replication, e.g. preventing viral binding and entry into cells
  - promoting opsonophagocytosis of extracellular bacteria, i.e. enhancing clearance by macrophages and neutrophils
  - activating the complement cascade
- $CD8^+$ T cells do not prevent but reduce, control and clear intracellular pathogens by:
  - directly killing infected cells (release of perforin, granzyme, etc.)
  - indirectly killing infected cells through antimicrobial cytokine release
- $CD4^+$ T cells do not prevent but participate to the reduction, control and clearance of extra- and intracellular pathogens by :
  - producing IFN-γ, TNF-α/-β, IL-2 and IL-3 and supporting activation and differentiation of B cells, $CD8^+$T cells and macrophages (Th1 cells)
  - producing IL-4, IL-5, IL-13, IL-6 and IL-10 and supporting B cell activation and differentiatio n (Th2 cells)

Table 2–2 Correlates of Vaccine-Induced Immunity

Vaccines	Vaccine type	Serum IgG	Mucosal IgG	Mucosal IgA	T cells
Diphtheria toxoid	toxoid	++	(+)		
Hepatitis A	killed	++			
Hepatitis B (HbsAg)	protein	++			
Hib PS	PS	++	(+)		
Hib glycoconjugates	PS-protein	++	++		
Influenza	killed, subunit	++	(+)		
Influenza intranasal	live attenuated	++	+	+	+ ($CD8^+$)
Measles	live attenuated	++			+ ($CD8^+$)
Meningococcal PS	PS	++	(+)		
Meningococcal conjugates	PS-protein	++	++		
Mumps	live attenuated	++			
Papillomavirus	VLPs	++	++		
Pertussis, whole cell	killed	++			
Pertussis, acellular	protein	++			+?($CD4^+$)
Pneumococcal PS	PS	++	(+)		
Pneumococcal conjugates	PS-protein	++	++		
Polio Sabin	live attenuated	++	++	++	
Polio Salk	killed	++	+		
Rabies	killed	++			
Rotavirus	live attenuated			++	
Rubella	live attenuated	++			
Tetanus toxoid	toxoid	++			
Tuberculosis (BCG)	live mycob				++($CD4^+$)
Typhoid PS	PS	+	(+)		
Varicella	live attenuated	++			+?($CD4^+$)
Yellow Fever	live attenuated	++			

PS : polysaccharide
VLP : virus-like-particle
Note : this table may not be exhaustive and only includes currently licensed vaccines.

responses.[6–8] The conjugation of bacterial PS to a protein carrier (e.g., glycoconjugate vaccines) provides foreign peptide antigens that are presented to the immune system and thus recruits antigen-specific $CD4^+$ Th cells in what is referred to as T-dependent antibody responses*.[9,10] A hallmark of T-dependent responses, which are also elicited by toxoid, protein, inactivated or live attenuated viral vaccines (Table 2–2), is to induce both higher-affinity antibodies and immune memory. In addition, live attenuated vaccines usually generate $CD8^+$ cytotoxic T cells. The use of live vaccines/vectors or of specific novel delivery systems (e.g. DNA vaccines) appears necessary for the induction of strong $CD8^+$ T cell responses. Most current vaccines mediate

their protective efficacy through the induction of vaccine-specific antibodies, whereas BCG-induced T cells produce cytokines that contribute to macrophage activation and control of *M. tuberculosis*.[11]

The induction of antigen-specific immune effectors (and/or of immune memory cells) by an immunization process does not imply that these antibodies, cells or cytokines represent surrogates—or even correlates—of vaccine efficacy. This requires the formal demonstration that vaccine-mediated protection is dependent—in a vaccinated individual—upon the presence of a given marker such as an antibody titer or a number of antigen-specific cells above a given threshold. Antigen-specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases[12] (Table 2–2). Passive protection may result from the physiological transfer of maternal antibodies (e.g., tetanus) or the passive administration of immunoglobulins or vaccine-induced hyperimmune serum (e.g., measles, hepatitis, varicella, etc.). Such antibodies may neutralize toxins in the periphery, at their site of production in an infected wound (tetanus) or throat (diphtheria). They may reduce binding or adhesion to susceptible cells/receptors and thus prevent viral replication (e.g., polio) or bacterial colonization (glycoconjugate vaccines against encapsulated bacteria) if present at sufficiently high titers on mucosal surfaces.[13] The neutralization of pathogens at mucosal surfaces is mainly achieved by the transudation of vaccine-induced serum IgG antibodies. It requires serum IgG antibody concentrations to be of sufficient affinity and abundance to result in 'protective' antibody titers in saliva or mucosal secretions. As a rule, such responses are not elicited by PS bacterial vaccines but achieved by glycoconjugate vaccines, which therefore prevent nasopharyngeal colonization in addition to invasive diseases.

Under most circumstances, immunization does not elicit sufficiently high and sustained antibody titers on mucosal surfaces to prevent local infection. It is only after having infected mucosal surfaces that pathogens encounter vaccine-induced IgG serum antibodies that neutralize viruses, opsonize bacteria, activate the complement cascade (Table 2–1) and limit their multiplication and spread, preventing tissue damage and thus clinical disease. That vaccines fail to induce sterilizing immunity is thus not an obstacle to successful disease control, although it represents a significant challenge for the development of specific vaccines such as against HIV-1.[14]

Current vaccines mostly mediate protection through the induction of highly specific IgG serum antibodies (Table 2–2). Under certain circumstances, however, passive antibody-mediated immunity is inefficient (tuberculosis). BCG is the only currently used human vaccine for which there is conclusive evidence that T cells are the main effectors.[11] However, there is indirect evidence that vaccine-induced T cells contribute to the protection conferred by other vaccines. $CD4^+$ T cells seem to support the persistence of protection against clinical pertussis in children primed in infancy, after vaccine-induced antibodies have waned.[15–18] Another example is that of measles immunization in 6-month-old infants. These infants fail to raise antibody responses because of immune immaturity and/or the residual presence of inhibitory maternal antibodies, but generate significant IFN-γ producing $CD4^+$ T cells.[19–21] These children remain susceptible to measles infection, but are protected against severe disease and death, presumably because of the viral clearance capacity of their vaccine-induced T cell effectors. Thus, prevention of infection may only be achieved by vaccine-induced antibodies, whereas disease attenuation and protection against complications may be supported by T cells even in the absence of specific antibodies. The understanding of vaccine immunology thus requires appraising how B and T cell responses are elicited, supported, maintained and/or reactivated by vaccine antigens.

From innate to adaptative immunity activation: the first steps after immunization

The induction of antigen-specific B and T cell responses requires their activation by specific antigen presenting cells (APC), essentially dendritic cells (DC) that must be recruited into the reaction. Immature DCs patrol throughout the body. When exposed to pathogens, they undergo a brisk maturation, modulate specific surface receptors and migrate towards secondary lymph nodes, where the induction of T and B cell responses occurs. The central role for mature DCs in the induction of vaccine responses reflects their unique capacity to provide both antigen-specific and costimulation signals to T cells, these 'danger signals' being required to activate naïve T cells.[22] The very first requirement to elicit vaccine responses is thus to provide sufficient danger signals through vaccine antigens and/or adjuvants* (Fig. 2–1), to trigger an inflammatory reaction that is mediated by cells of the innate immune system.[23]

DCs, monocytes and neutrophils express a set of receptors directed against evolutionarily conserved pathogen patterns that are not contained in self-antigens and thus readily identified as 'danger.' Through these receptors, among which Toll-like receptors* play an essential role (Table 2–3),[24] these host cells sense the potential danger when they encounter a pathogen and become activated (Fig. 2–2). They modulate the expression of their surface molecules and produce proinflammatory cytokines and chemokines.[25] This results into the extravasation and attraction of monocytes, granulocytes and natural killer cells, and generates an inflammatory microenvironment (Fig. 2–1) in which monocytes differentiate into macrophages and immature dendritic cells become activated.[26] This activation modifies the expression of homing receptors at their surface and triggers DC migration towards the draining lymph nodes (Fig. 2–2). In the absence of danger signals, DCs remain immature : upon contact with naïve T cells, T cells do not differentiate into immune effectors but into regulatory $CD4^+$ T cells which maintain immune tolerance.[2]

Live viral vaccines efficiently trigger the activation of the innate immune system, presumably through pathogen-associated signals (such as viral RNA) allowing their recognition by pattern recognition receptors (Table 2–3). Following injection, viral particles rapidly disseminate throughout the vascular network and reach their target tissues. This pattern is very similar to that occurring after a natural infection, including the initial mucosal replication stage for vaccines administered through the nasal/oral routes. Following the administration of a live viral vaccine and its dissemination, dendritic cells are thus activated at multiple sites, migrate towards the corresponding draining lymph nodes and launch multiple foci of T and B cell activation. This provides a first explanation to the generally higher immunogenicity of live versus non-live vaccines (Table 2–4). Another consequence of this early diffusion pattern is that the site and route of injection of live viral vaccines are of minor importance: for example, the immunogenicity and reactogenicity of measles vaccine is similar following intramuscular or subcutaneous injection.[27] Live bacterial vaccines, such as BCG, multiply both at the site of injection, where they generate the induction of a prolonged inflammatory reaction, and at distance—with preponderance for local draining lymph nodes.

Non-live vaccines, whether containing proteins, polysaccharides, glycoconjugates or inactivated microorganisms (Table 2–2), may still contain pathogen recognition patterns capable of initiating innate responses.[28] In the absence of microbial replication, however, vaccine-induced activation remains more limited, both in time and space. Non-live vaccines essentially activate innate responses at their site of injection

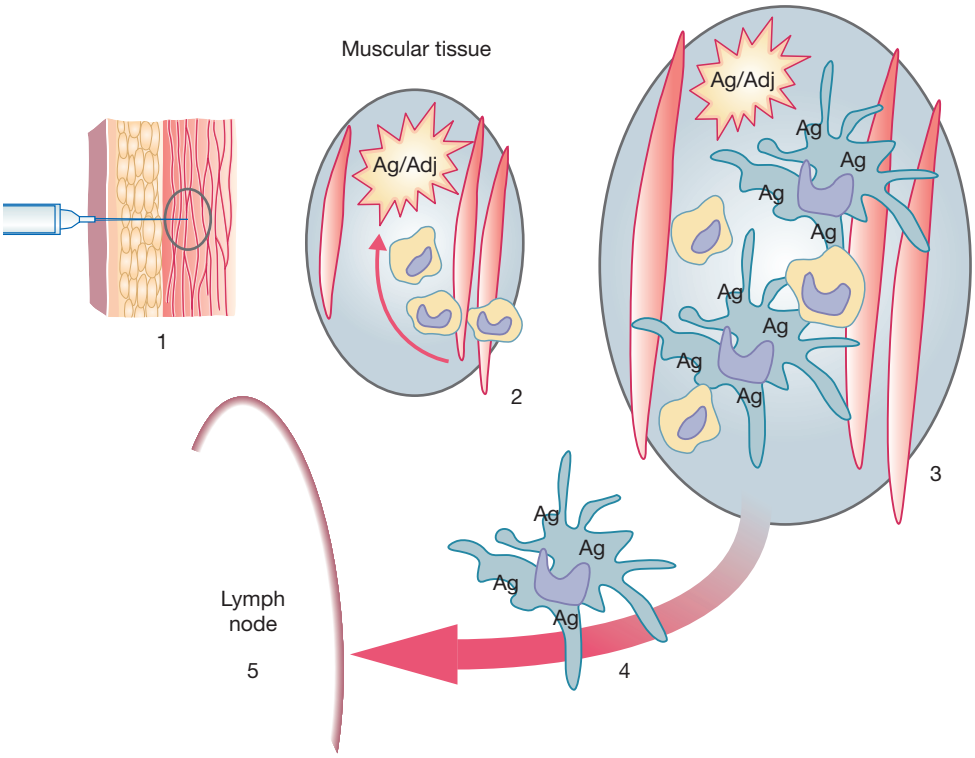

Figure 2–1 Initiation of a vaccine response.

Following injection (1), the pathogen-associated patterns contained in vaccine antigens attract dendritic cells, monocytes and neutrophils that patrol throughout the body (2). If vaccine antigens/adjuvants elicit sufficient 'danger signals,' this activates monocytes and dendritic cells (3) which changes their surface receptors and induces their migration along lymphatic vessels (4), to the draining lymph nodes (5) where the activation of T and B lymphocytes will take place.

Table 2–3 Recognition of Vaccine Determinants by Pattern Recognition Receptors

Receptors	Ligands	Demonstrated ligands in vaccine antigens
TLR1	Certain bacterial lipoproteins	
TLR2	Peptidoglycan, lipoproteins, glycolipids, lipopolysaccharide	BCG, Hib-OMP, pneumococcal PS
TLR3	Viral double-stranded RNA	BCG, pneumococcal PS, HPV-VLPs
TLR4	Bacterial lipopolysaccharides	
TLR5		
TLR6	Bacterial flagellins	
TLR7	Lipotechoic acid, lipopeptides	Yellow-fever, live attenuated influenza, whole cell influenza
TLR8	Single-stranded RNA	
TLR9		Yellow-fever
TLR10	Single-stranded RNA	Yellow-fever
NOD1, NOD2	CpG oligonucleotides Unknown Peptidoglycans	Pneumococcal PS

(Fig. 2–1). Their site and route of administration are thus more important. The high number of DCs in the derma allows a marked reduction (e.g., 10-fold) of the antigen dose in intradermal immunization, an advantage that is applied to the prevention of rabies in many countries. It however generally results in lower vaccine antibody responses[29], which might be associated to the preferential induction of Th1 responses by skin DCs. Patrolling DCs are also numerous in the well-vascularized muscles, which is the preferred route of injection for most vaccines. They are fewer in adipose tissues, such that subcutaneous injections may be less effective than intramuscular injections under conditions of limited immunogenicity, such as for adult immunization against hepatitis B.[30]

Despite many efforts, immunization through the mucosal route is currently limited to a few live vaccines. The extreme difficulty in producing non-live mucosal vaccines reflects the need to overcome a large number of physical, immunological and chemical barriers, which requires the use of strong adjuvants.[31] This is not trivial, as unfortunately illustrated by the association of a novel adjuvanted inactivated intranasal influenza vaccine with Bell's palsy.[32]

Following their activation, DCs migrate towards the local draining lymph nodes, e.g., towards the axillary and the inguinal area following deltoid and quadriceps injection, respectively. That primary immune responses to non-live vaccines are essentially focal and unilateral is likely to contribute to the fact that the simultaneous administration of several distinct vaccines may take place without immune interference if vaccines are administered at sites draining into distinct lymph node areas. Most non-live vaccines require their formulation with specific

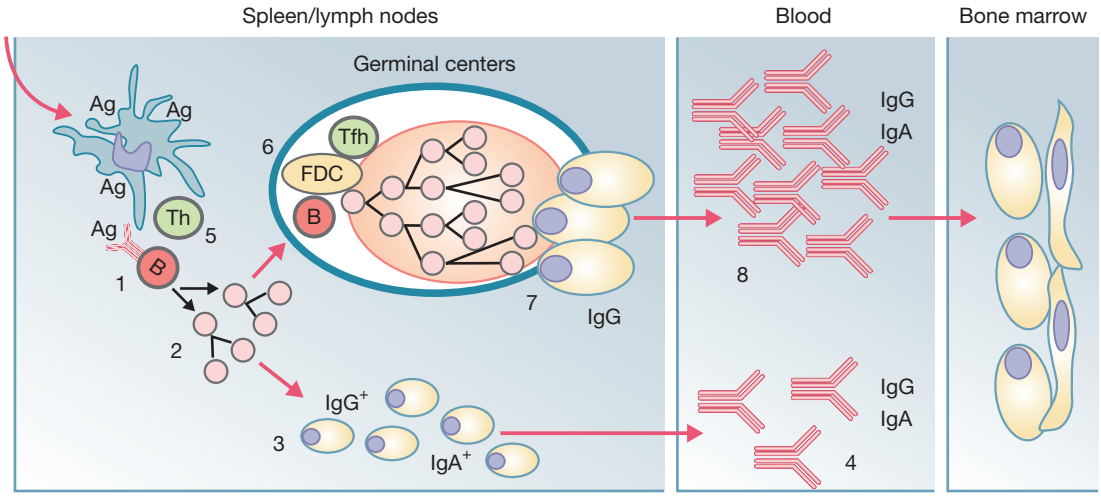

Figure 2–2 Extrafollicular and germinal center responses to protein antigens. In response to a protein antigen reaching lymph nodes or spleen, B cells capable of binding to this antigen with their surface immunoglobulins (1) undergo a brisk activation. In an extrafollicular reaction (2), B cells rapidly differentiate in plasma cells (3) that produce low-affinity antibodies (of the IgM +/– IgG/IgA isotypes) that appear at low levels in the serum within a few days after immunization (4). Antigen-specific helper T cells (5) that have been activated by antigen-bearing dendritic cells trigger some antigen-specific B cells to migrate towards follicular dendritic cells (FDCs) (6), initiating the germinal center (GC) reaction. In GCs, B cells receive additional signals from follicular T cells (Tfh) and undergo massive clonal proliferation, switch from IgM towards IgG, IgA or IgE, undergo affinity maturation (7) and differentiate into plasma cells secreting large amounts of antigen-specific antibodies (8). At the end of the GC reaction, a few plasma cells exit nodes/spleen and migrate to survival niches mostly located in the bone marrow, where they survive through signals provided by supporting stromal cells (9).

Table 2–4 Determinants of Primary Vaccine Antibody Responses in Healthy Individuals

Determinants	Mechanisms (presumed)
Vaccine type	
Live vs inactivated	Higher intensity of innate responses, higher antigen content following replication and more prolonged antigen persistence generally result into higher Ab responses to live than inactivated vaccines.
Protein vs polysaccharide	Recruitment of T cell help and induction of GCs results into higher Ab responses to protein or glycoconjugate than to PS vaccines.
Adjuvants	Modulation of antigen delivery and persistence (depot or slow-release formulations) or enhancement of Th responses (immunomodulator) may support or limit Ab responses.
Antigen nature	
Polysaccharide antigens	Failure to induce GCs limit immunogenicity.
Protein antigens	Inclusion of epitopes readily recognized by B cells (B cell repertoire), inclusion of epitopes readily recognized by follicular helper T cells, elicitation of efficient follicular T cell help and the capacity of antigen to associate/persist in association to FDCs result into higher Ab responses.
Antigen dose	As a rule, higher Ag doses increase the availability of Ag for B / T cell binding and activation, as well as for association with FDCs.
Vaccine schedule	
Interval between doses	A 3 week minimal interval between primary doses avoids competition between successive waves of primary responses.
Genetic determinants	The capacity of Ag epitopes to associate to a large panel of MHC molecules increases the likelihood of responses in the population. MHC restriction may limit T cell responses. Gene polymorphisms in molecules critical for B and T cell activation/differentiation are likely to affect Ab responses.
Environmental factors	Mostly yet identified.
Age at immunization	Early life immune immaturity or age-associated immune senescence.

adjuvants to include danger signals and trigger a sufficient activation of the innate system. These adjuvants may be divided into two categories: delivery systems that prolong the antigen deposit at site of injection, recruiting more DCs into the reaction, and immune modulators that provide additional differentiation and activation signals to monocytes and DCs.[26] Although progress is being made, none of the adjuvants currently in use trigger the degree of innate immune activation that is elicited by live vaccines, whose immune potency far exceed that of non-live vaccines.

Vaccine antibody responses

How are primary antibody responses elicited?

B cells are activated in the lymph nodes that have been reached by vaccine antigens, upon diffusion and/or in association to migrating DCs. Protein antigens activate both B and T cells, which results in the induction a highly efficient B cell differentiation pathway through specific structures (germinal centers, GCs) in which antigen-specific B cells proliferate and differentiate into antibody-secreting plasma cells or memory B cells. Polysaccharide antigens that fail to activate T cells into the response do not trigger GCs, such that they elicit weaker and shorter antibody responses, and no immune memory.

T-dependent responses to protein antigens

The extrafollicular reaction

Naïve B cells generated in the bone marrow circulate until they encounter a protein antigen to which their specific surface IgM receptor may bind. Antigen binding initiates B cell activation and triggers the upregulation of CCR7, a chemokine receptor that drives antigen-specific B cells towards the outer T cell zone of secondary lymphoid tissues.[33] At this location, vaccine antigen-specific B cells are exposed to recently (<24 h) activated DCs and T cells that have up-regulated specific surface molecules and thus provide B cell activating signals. This T cell help rapidly drives B cell differentiation into Ig secreting plasma cells that produce low-affinity germline antibodies, in what is called the extrafollicular reaction (Figs 2–2 and 2–3).[34]

Immunoglobulin class-switch recombination from IgM towards IgG, IgA or IgE occurs during this differentiation of B cells, through the upregulation of the activation-induced deaminase (AID) enzyme. Both $CD4^+$ Th1 and Th2 cells exert essential helper functions during the extrafollicular differentiation pathway, and the engagement of their CD40L molecules with CD40 on B cells may skew class-switch recombination into particular Ig classes and subclasses. In rodents, IFN-γ producing Th1 T cells promote a switch towards IgG2a, whereas Th2 cells essentially support the generation of IgG1 and IgE (via IL-4) and IgG2b and IgG3 (via TGF-β).[35] The situation is less clear-cut in humans, where IgG1 antibodies frequently predominate regardless of the polarization of T cell help. The extrafollicular reaction is rapid, and IgM and low-level IgG antibodies appear in the blood a few days after primary immunization (Figs 2–2 and 2–3). These antibodies are of germline affinity, as there is no hypermutation/selection process during the extrafollicular reaction. This extrafollicular reaction is short-lived, as most cells die from apoptosis within a few days. Consequently, it probably plays a minor role in vaccine efficacy.

The germinal center reaction

Antigen-specific B cells that receive sufficient help from antigen-specific T cells proliferate in specialized structures called germinal centers (GCs) in which they differentiate into plasma cells. The induction of GCs is initiated as a few antigen-specific activated B cells up-regulate their expression of CXCR5 and migrate towards B cell follicles, being attracted there by CXCL13-expressing follicular dendritic cells (FDCs). FDCs play an essential role in B cell responses: they attract antigen-specific B and T cells and capture/retain antigen for extended periods. B cells that are attracted by Ag-bearing FDCs become the founders of GCs (Fig. 2–2). Receiving additional activation and survivals signals from both FDCs and follicular T cells, they undergo massive clonal proliferation—such that each GC is constituted by the progeny of a single antigen-specific B cell. This intense proliferation is associated to two major events: Ig class-switch recombination from IgM towards IgG, IgA or IgE, and maturation of the affinity of B cells for their specific antigen.

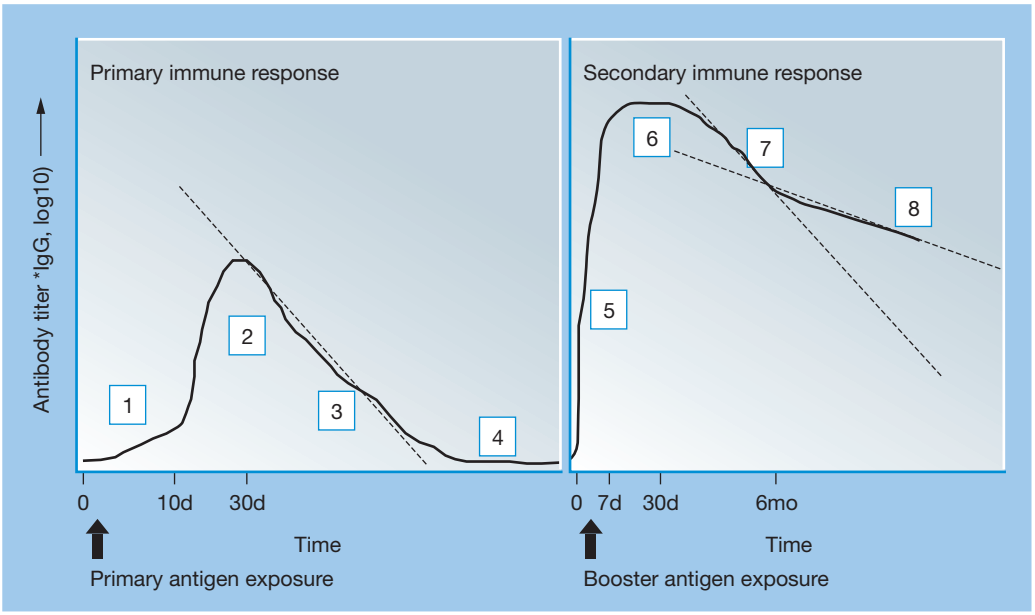

Figure 2–3 Correlation of antibody titers to the various phases of the vaccine response. The initial antigen exposure elicits an extrafollicular response (1) that results in the rapid appearance of low IgG antibody titers. As B cells proliferate in germinal centers and differentiate into plasma cells, IgG antibody titers increase up to a peak value (2) usually reached 4 weeks after immunization. The short life span of these plasma cells results in a rapid decline of antibody titers (3), which eventually return to baseline levels (4). In secondary immune responses, booster exposure to antigen reactivates immune memory and results in a rapid (<7 days) increase (5) of IgG antibody titer. Short-lived plasma cells maintain peak Ab levels (6) during a few weeks—after which serum antibody titers decline initially with the same rapid kinetics as following primary immunization. Long-lived plasma cells that have reached survival niches in the bone marrow continue to produce antigen-specific antibodies, which then decline with slower kinetics. Note: this generic pattern may not apply to live vaccines triggering long-term IgG antibodies for extended periods of time.

This results into the higher production of antibodies of a higher antigen binding capacity (Fig. 2–3).

The maturation of B cell affinity results from an extensive somatic hypermutation process within the variable-region segments of immunoglobulin genes. In most B cells, this stochastic process results inadvertently into a decline of the affinity of B cell Ig for antigen. In a small minority of B cells, however, the introduction of mutations in their Ig genes increases the affinity of their surface IgG for antigen. This enables these B cells to efficiently compete for binding to the small amounts of vaccine antigens that are associated to the surface of FDCs (Fig. 2–2). B cells process these vaccine antigens into small peptides that they display at their surface through MHC class II molecules. MHC-peptides complexes thus become available for binding by a specific subset of $CD4^+$ T cells, follicular helper T cells (Tfh).[36] These Tfh, which express CXCR5, have migrated towards CXCL13-expressing FDCs. Differing from Th1 and Th2 cells by their chemokine receptors, transcription factors, surface markers and interleukins,[36] they are uniquely equipped to provide a most efficient B cell help through a series of costimulation molecules, including CD40L, ICOS, the IL-10 B cell growth factor and IL-21.[36] The cellular interactions between antigen-specific GC B cells, antigen-bearing FDCs and antigen-specific Tfh cells (Fig. 2–2) result in the proliferation, survival and selection of B cells that have the highest possible antigen-specific affinity. They also provide the signals required for the subsequent differentiation of GC B cells either towards plasma cells secreting large amounts of specific antibodies or towards memory B cells.

The development of this GC reaction requires a couple of weeks, such that hypermutated IgG antibodies to protein vaccine antigens first appear in the blood 10–14 days after priming (Fig. 2–3).[37] Feedback mechanisms terminate GC reactions within 3–6 weeks, a period during which a large number of antigen-specific plasma cells will have been generated. It is the magnitude of GC responses, i.e., the quality of DC, B cell, Tfh cell and FDC interactions, which controls the intensity of B cell differentiation into plasma cells, and thus the peak of IgG vaccine antibody reached within 4–6 weeks after primary immunization (Fig. 2–3).

T-independent responses to polysaccharide antigens

Bacterial (*S. pneumoniae, N. meningitidis, H. influenzae, S. typhi*) polysaccharide antigens released from the injection site essentially reach the marginal zone of the spleen/nodes through the blood, an area that is equipped by macrophages exhibiting a unique set of scavenger receptors. There, PS bind to marginal zone B cells and their repetitive structure cross-links the Ig receptors at the B cell surface.[34] This activates marginal zone B cells in extrafollicular foci (Fig. 2–4).[34] During the week following immunization, B cells differentiate into plasma cells, undergo some degree of isotype switching from IgM to IgG/IgA and—in rodents—rapidly produce essentially non-mutated, low-affinity, germline antibodies. Thus, PS vaccines are generally known as triggering T-independent responses characterized by the induction of moderate titers of low-affinity antibodies, and the absence of immune memory.

In humans, PS immunization does generate the production of intermediate-affinity IgG antibodies bearing some somatic mutations in their variable regions.[38,39] The production of mutated antibodies is not expected during a T-independent immune response, as somatic mutations essentially take place in germinal centers (GC). One hypothesis is that PS immunization activates 'memory' B cells that have been previously primed by cross-reacting PS bacterial antigens somehow linked to protein moieties—and thus eliciting GC responses.[40] An alternative possibility is that the IgM^+, IgD^+, $CD27^+$ 'memory' B cells that appear in the blood in response to PS immunization may be re-circulating splenic marginal zone B cells.[41] These cells would diversify their Ig receptor to a certain extent in the absence of cognate T-B interaction.[41] This hypothesis is concordant with the fact that bacterial PS vaccines are poorly immunogenic in young children, i.e., prior to the maturation of the spleen marginal zone.[42,43]

After their differentiation in the extrafollicular pathway, PS-specific plasma cells move towards the red pulp of the spleen (Fig. 2–4) where they persist for some time, prior to their death by apoptosis and the waning of corresponding antibody responses after a few months. As PS antigens do not induce GCs, bona fide memory B cells are not elicited. Consequently, subsequent re-exposure to the same PS results into a repeat primary response that follows the same kinetics in previously vaccinated as in naïve individuals.[44] Revaccination with certain bacterial PS—of which group C *N. meningitidis* is a prototype—may even induce lower antibody responses than the first immunization, a phenomenon referred to as hyporesponsiveness and whose molecular and cellular bases are not yet fully understood.[45,46]

Which are the determinants of primary vaccine antibody responses?

Numerous determinants modulate the intensity of vaccine-induced GCs—and thus of peak antibody responses (Table 2–5). The main determinants are the nature of the vaccine antigen

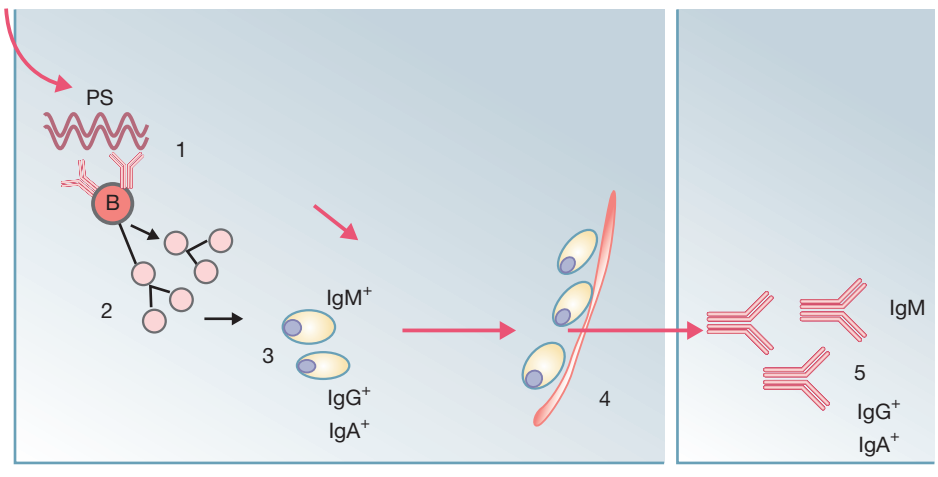

Figure 2–4 Extrafollicular B cell responses to polysaccharide antigens. B cells use their specific Ig surface receptors (1) to bind to the repetitive structures of polysaccharides reaching the marginal zone of spleen/nodes. In the absence of antigen-specific T cell help, B cells are activated, proliferate (2) and differentiate in plasma cells (3) without undergoing affinity maturation in germinal centers. These plasma cells migrate towards the red pulp of the spleen (4) where they survive for a few weeks / months, secreting low levels of low affinity IgM, IgG or IgA antibodies (5).

Table 2–5 Determinants of the Duration of Vaccine Antibody Responses in Healthy Individuals

Determinants	Mechanisms (presumed)
Vaccine type	
Live vs inactivated	Live vaccines generally induce more sustained Ab responses, presumably through Ag persistence within the host.
Polysaccharide antigens	Failure to generate GCs limits the induction of memory responses and of high-affinity long-live plasma cells.
Vaccine schedule	
Interval between primary doses	A minimal interval of 3 weeks between primary doses allows development of successive waves of Ag-specific primary responses without interference.
Interval before boosting	A minimal interval of 4 months between priming and boosting allows affinity maturation of memory B cells, and thus higher secondary responses.
Age at immunization	Early life immune immaturity and age-associated immunosenescence limit the induction/persistence of long-live plasma cells
Environmental factors	?

and its intrinsic immunogenicity. As an example, tetanus toxoid is intrinsically a stronger immunogen than diphtheria toxoid, which becomes apparent when immunocompetence is more limited, such as in preterm infants.[47] Whether this reflects a higher capacity of tetanus toxoid to provide antigenic epitopes that may be bound by naïve B cells, to generate cognate T cell help for B cells, and/or to associate to FDCs is unknown.

The drastically distinct outcomes of immunization with plain bacterial PS or with protein-conjugated glycoconjugates highlight the differences between the extrafollicular and the GC reactions. It is only when capsular PS are conjugated to a protein carrier driving effective Th differentiation that PS-specific B cells are driven towards GC responses, receive optimal cognate help from carrier-specific Tfh cells and differentiate into higher-affinity antibody producing cells, longer-lived plasma cells and/or memory B cells. Protein antigens exhibit markedly distinct carrier properties—regardless of their capacity to induce B and Th cell responses.[48] That these differences may reflect differences in Tfh induction is an interesting hypothesis which is supported by the enhanced immunogenicity of a synthetic polyepitope carrier containing optimal Th epitopes.[49] The limited number of potent carrier proteins implies that an increasing number of conjugate vaccines rely on the same molecules (e.g., CRM_{197}, tetanus or diphtheria toxoids), with the risk of limiting anti-PS responses to individual conjugate vaccines (carrier-mediated epitope suppression).[50] This phenomenon may be abrogated by replacing full-length proteins by peptides lacking B-cell epitopes,[51] suggesting that carrier-mediated epitope suppression essentially reflects the competition of carrier- and PS-specific B cells for activation/differentiation signals and factors.

Another determinant of the magnitude of primary vaccine antibody responses (Table 2–5) is the use of an optimal dose of vaccine antigen, which may only be experimentally determined. As a rule, higher doses of non-live antigens—up to a certain threshold—elicit higher primary antibody responses. This may be particularly useful when immunocompetence is limited, e.g., for hepatitis B immunization of dialysis patients.[52,53] Remarkably, a limiting dose of vaccine antigen may restrict primary antibody responses but increase B cell competition for FDC-associated antigens, and thus result into a more stringent selection of higher affinity GC B cells and into stronger secondary responses (see below). Little is yet known on factors which support or limit the affinity maturation process. Interestingly, carrier proteins[54] and adjuvants may modulate the affinity maturation process, as recently observed following the addition of CpG oligonucleotides to an alum-adsorbed hepatitis B vaccine.[55]

The nature of the vaccine directly influences the activation of innate immunity and thus vaccine responses.[26] The strongest antibody responses are generally elicited by live vaccines that better activate innate reactions and thus better support the induction of adaptative immune effectors. Non-live vaccines frequently require formulation in adjuvants, of which aluminum salts are particularly potent enhancers of antibody responses, and thus included in a majority of currently available vaccines. This is likely to reflect their formation of a deposit from which antigen is slowly de-absorbed and released, extending the duration of B and T cell activation, as well as the preferential induction of IL-4 by aluminum-exposed macrophages.[56]

Genetic determinants directly influence the vaccine antibody responses of healthy individuals, as exemplified by twin studies.[57–61] Apart from MHC restriction, few genetic determinants of vaccine antibody responses have yet been identified.[58] Immune competence obviously affects vaccine antibody responses, which are limited at the two extremes of life (see below), by acute or chronic diseases, by acute or chronic stress and by a variety of factors affecting innate and/or B and T cell immunity.

Very few non-live vaccines induce high and sustained antibody responses after a single vaccine dose, even in healthy young adults. Primary immunization schedules therefore usually include at least two vaccine doses, optimally repeated at a minimal interval of 3–4 weeks to generate successive waves of B cell and GC responses. These priming doses may occasionally be combined into a single 'double' dose, such as for hepatitis A or B immunization.[62,63] In any case, however, vaccine antibodies elicited by primary immunization with non-live vaccines eventually wane (Fig. 2–3).

What controls the persistence of vaccine antibody responses?

Antigen-specific plasma cells elicited in spleen/nodes after immunization only have a short life span, such that vaccine antibodies rapidly decline during the first few weeks and months after immunization. A fraction of plasma cells that differentiated into GCs however acquire the capacity to migrate towards long-term survival niches mostly located within the bone marrow (BM), from where they may produce vaccine antibodies during extended periods.[64]

Some GC-induced plasma cells are attracted toward the BM compartment by specific BM stromal cells that provide the

signals required for their long-term survival.[65] In such BM niches, plasma cell survival and antibody production may persist for years. Whether the persistence of vaccine-induced plasma cells reflects the long-term persistence of the plasma cells that were initially generated, or the maintenance of a BM reservoir of plasma cells through homeostatic mechanisms, is yet undefined.[66] Regardless of the exact mechanisms supporting BM plasma cell survival, the duration of antibody responses is proportional to the number of long-lived plasma cells generated by immunization: in absence of subsequent antigen exposure, antibody persistence may be reliably predicted by the antibody titers that are reached 6–12 months after immunization, i.e. after the end of the short-term plasma cell response (Fig. 2–3). This is illustrated by the accuracy of mathematical models predicting the kinetics of anti-HBsAg[67] or anti-hepatitis A[68] antibodies.

A few determinants of the persistence of vaccine antibody responses (Table 2–5) have been identified.[66] The nature of the vaccine plays a crucial role: only live attenuated viral vaccines induce antibody responses that persist for several decades, if not life-long, in absence of subsequent antigen exposure and reactivation of immune memory. This could reflect the *in vivo* persistence of viral antigens that continuously trigger B cell responses, although other mechanisms may be at play. In contrast, the shortest antibody responses are elicited by PS antigens, which fail to trigger GC responses and thus do not elicit high-affinity plasma cells capable of reaching the BM survival niches. Vaccine schedules also control antibody magnitude and persistence. Closely spaced (1–2 weeks) primary vaccine doses may be administered when a rapid induction of protection is desirable, e.g., prior to travel. However, this raises less persisting responses than when the same number of vaccine doses are given at longer (1–2 months) intervals,[69,70] reflecting the generation of fewer post-GC B cells capable of long-term survival and thus requiring later boosting.

Age at immunization also modulates vaccine antibody persistence, which is shorter at the two extremes of life (see below). Certain disease conditions may also limit the persistence of vaccine antibody responses because of an enhanced catabolism (malaria) or the loss of antibodies in the urinary or digestive tracts. An interesting recent observation is that human immunization with tetanus toxoid results into a transient release of resident BM plasma cells into the blood,[71] suggesting the existence of a competition for BM survival niches between newly induced and BM resident plasma cells. The identification of the mechanisms that support or limit the persistence of vaccine antibody responses represents a major challenge for vaccinologists, as resources are lacking for most immunization programs throughout the world to include the booster doses otherwise required to maintain vaccine efficacy.

Which are the hallmarks of B cell memory responses?

Memory B cells are generated during primary responses to T-dependent vaccines. They do not produce antibodies, i.e., do not protect, unless re-exposure to antigen drives their differentiation into antibody producing plasma cells. This reactivation is a rapid process, such that booster responses are characterized by the rapid increase to higher titers of antibodies that have a higher affinity for antigen than antibodies generated during primary responses (Table 2–6).

With the possible exception of responses to live viral vaccines, vaccine antibody responses are deemed to wane and eventually decline below protective thresholds, unless repeat antigen exposure reactivates immune memory. Memory B cells are generated in response to T-dependent antigens, during the GC reaction, in parallel to plasma cells (Fig. 2–5). At their exit of GCs, memory B cells acquire migration properties towards extrafollicular areas of the spleen and nodes.[72] This migration occurs through the blood, in which post-immunization memory B cells are transiently present on their way towards lymphoid organs.

It is essential to understand that memory B cells do not produce antibodies—i.e., they do not protect. Their participation to vaccine efficacy requires an antigen-driven proliferation and differentiation process.[72] This reactivation may occur in response to endemic or frequent pathogens, to colonizing or cross-reacting microorganisms ('natural boosters'), or to booster immunization. The antigen-driven activation of memory B cells results in their rapid proliferation and differentiation into plasma cells that produce very large amounts of higher-affinity antibodies.[72] As the affinity of surface Ig from memory B cells is increased, their requirements for reactivation are lower

Table 2–6 Hallmarks of Memory B cell Responses

Are only generated during T-dependent responses inducing GC responses.
Memory B cells are resting cells that do not produce antibodies.
Memory B cells undergo affinity maturation during several (4–6) months.
Memory B cells rapidly (days) differentiate into antibody-secreting plasma cells upon re-exposure to antigen.
Memory B cells differentiate into PCs that produce high(er) affinity antibodies than primary plasma cells.

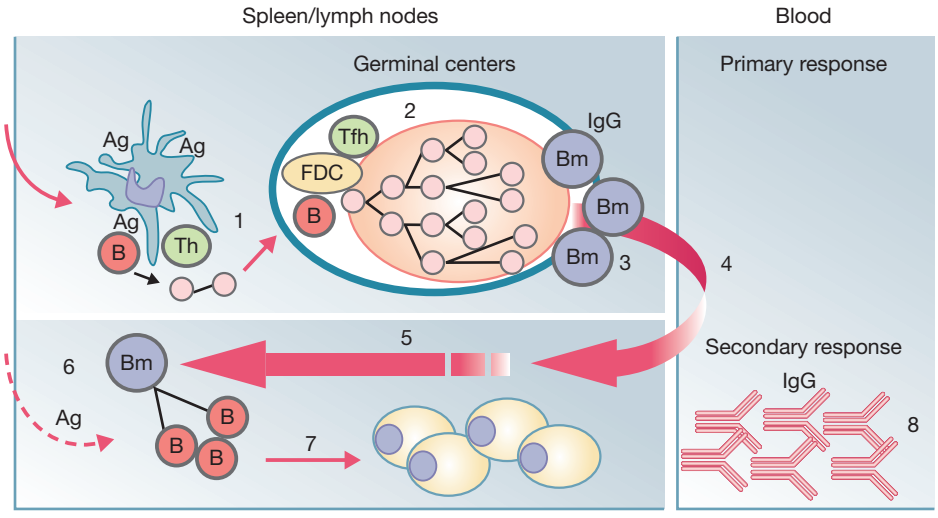

Figure 2–5 Generation of B cell memory responses. Memory B cells are generated in response to T-dependent antigens (1), during the GC reaction (2), in parallel to plasma cells. At their exit of GCs, these B cells do not differentiate into antibody-secreting plasma cells but in memory B cells (3) that transiently migrate through the blood (4) towards the extrafollicular areas of spleen and nodes (5). They persist there as resting cells until re-exposed to their specific antigens (6). Upon secondary antigen exposure, memory B cells readily proliferate and differentiate into plasma cells (7) secreting large amounts of high-affinity antibodies that may be detected in the serum (8) within a few days after boosting.

than for naïve B cells: memory B cells may thus be recalled by lower amounts of antigen and without CD4+ T cell help. Antigen-specific memory cells generated by primary immunization are much more numerous (and better fit) than naïve B cells initially capable of antigen recognition. Thus, a first hallmark of memory responses (Table 2–6) is to generate significantly higher antibody levels than primary immunization. Should this not be the case, the effective generation of memory B cells should be questioned.[72]

The reactivation, proliferation and differentiation of memory B cells occur without requiring the induction and development of GC responses. This process is thus much more rapidly completed than that of primary responses. A window of 4–7 days after *Haemophilus influenzae b* PS immunization was reported as sufficient for high levels of PS-specific vaccine antibodies to appear in the blood of previously primed infants.[73] The rapidity with which antigen-specific antibodies appear in the serum is thus another hallmark of secondary responses (Table 2–6). Slower kinetics suggests that memory B cell induction, persistence and/or reactivation may have been suboptimal.

Another hallmark of memory B cells is to display and secrete antibodies with a markedly higher affinity than those produced by primary plasma cells, as a result of somatic hypermutation and selection.[72] The affinity maturation process which is initiated within the GCs extends during several months after the end of the GC reaction. Consequently, vaccine antibodies with higher than baseline avidity (defined as the sum of epitope-specific affinities) for antigen are only induced when sufficient time has elapsed after priming.[62,74,75] A 'classical' prime-boost immunization schedule is thus to allow 4–6 months to elapse between priming and booster doses, hence the generic '0–1–6 months' schedule. Secondary antigen exposure (Table 2–6) thus results in the production of higher affinity antibodies than primary responses.[76] To note, this may not be the case when 'natural' priming, e.g., through cross-reactive bacteria, has taken place prior to immunization.

Which are the determinants of B cell memory responses?

The factors that drive the differentiation of antigen-specific GC B cells towards either plasma cells or memory B cells are yet poorly understood.[64] In response to protein antigens, both cell populations are generated in the same GCs, and their differentiation pathway only differs late in the GC reaction. As a rule, factors enhancing plasma cell differentiation and primary antibody responses therefore also support the induction of memory B cells (Table 2–7). Post-booster antibody titers are therefore higher in individuals with stronger primary responses. As an example, higher post-booster anti-HBsAg responses are observed in individuals with high (e.g. = 100 UI/l) rather than intermediate (10–99 UI/l) anti-HBsAg post-primary responses.[77,78] This is likely to reflect the induction of a larger pool of antigen-specific memory B cells. An interesting question is whether this may confer specific advantages in terms of protection: the protective threshold of serum antibodies could be reached more rapidly upon the reactivation of a larger number of memory B cells.

The dose of antigen is an important determinant of memory B cell responses (Table 2–7). At priming, higher antigen doses generally favor the induction of plasma cells, whereas lower doses may preferentially drive the induction of immune memory.[79] Thus, a lower antigen content may be preferred if the rapid induction of protection is not required. Closely spaced primary vaccine doses may also be beneficial for early post-primary antibody responses but not for post-booster antibody responses, as illustrated with meningococcal group C glycoconjugates.[80] As a rule, accelerated schedules in which a 4–6 months window is not included between priming and boosting result into significantly lower booster responses[62] (Table 2–7). At time of boosting, a higher antigen content raises stronger booster responses, presumably by recruiting more memory B cells into the response. This is illustrated by higher antibody responses of children primed with a glycoconjugate vaccine and boosted with PS (20–50 μg of PS) than glycoconjugate (1–3 μg of PS) vaccines.[81,82]

Residual titers of vaccine antibodies present at time of boosting directly influence vaccine antibody responses. As a rule, secondary responses to live attenuated viral vaccines are minimal, as pre-existing antibodies mostly neutralize the vaccine load prior to its in vivo replication. Consequently, even multiple doses of live attenuated vaccines remain without undesirable effects. Responses to non-live vaccines are also negatively influenced by residual vaccine antibody titers. This may reflect the formation of antigen-antibody complexes which reduce the load of antigen available for B cell binding and/or antibody-mediated negative feedback mechanisms acting directly on B cells. Consequently, individuals with residual antibodies to a given antigen may only show a limited increase of their antibody responses—such that vaccine responses are better described by the proportion of individuals above a given threshold than by those showing a 2- or 4-fold increase of their antibody titers.

The persistence of memory B cells is of utmost importance for long-term vaccine efficacy. Antigen persistence (Table 2–7) contributes to the duration of immune memory,[83] probably by extending the period during which antigen remains available for memory B cell induction and reactivation. This is likely to contribute to the extended (indefinite?) memory to live attenuated vaccines, recently exemplified by repeat administration of smallpox vaccines decades after priming.[84]

Table 2–7 Determinants of Secondary B cell Responses

Determinants	Mechanisms (presumed)
Post-primary antibody titers	As plasma cells and memory responses are generated in parallel in GCs, higher post-primary Ab titers reflect stronger GC reactions and generally predict higher secondary responses.
Residual antibodies at boosting	Neutralization of live viral vaccines; negative feedback mechanisms on non-live vaccines.
Lower antigen dose at priming	A limited quantity of antigen may induce B cell differentiation away from PCs, towards memory B cells (?).
Longer intervals before boosting	A minimal interval of 4–6 months is required for optimal affinity maturation of memory B cells.
Higher antigen dose at boosting	A higher availability of antigen may drive higher numbers of memory B cells into differentiation.
Antigen availability	
Exogenous exposure	Exposure to exogenous antigens may reactivate or favor the persistence of memory B cells.
In vivo persistence	Antigen persistence may reactivate or favor the persistence of memory B cells.

Fortunately, memory B cells survive for prolonged periods (e.g., several decades) even in the absence of re-exposure to antigen.[85] It has been suggested that memory B cells undergo a certain degree of homeostatic polyclonal activation.[86] Although this does not appear sufficient to maintain antibody responses, i.e., to drive their differentiation to Ig secreting cells, it may contribute to their persistence.

The demonstration of the persistence of memory B cells long after vaccine antibodies have eventually disappeared, and of their brisk reactivation upon antigen exposure, has direct consequences for immunization programs. First, it implies that an immunization schedule should never be started 'all over again'—but continued where interrupted, regardless of the duration of the interruption. Consequently, regular booster doses are not required to maintain immune memory during low-risk periods, which has direct implications for travellers who may simply need a single booster dose prior to departure. Second, it implies that certain immunization schedules may not need to include booster doses, should exposure provide regular natural boosters. Importantly, however, successful immunization programs may eventually reduce opportunities for natural boosters and consequently modify booster requirements. This issue will doubtlessly be an area of intense investigation in the next decades. Last, the long-term persistence of immune memory implies that booster vaccine doses may not be needed in situations where the reactivation of immune memory by offending pathogens is sufficiently rapid and effective to interrupt microbial invasion.

Immune memory and vaccine-induced protection: a race between reactivation and microbial invasion?

All existing vaccines, with the exception of T-independent PS, induce immune memory. Nevertheless, vaccine efficacy may be short-term, as illustrated following infant immunization against group C meningococcus.[87] Demonstration of priming—or 'boostability'—is therefore not a surrogate marker for long-term vaccine efficacy. This requires identifying the determinants that contribute—or limit—the persistence of vaccine efficacy.

It is generally considered that protection by toxoid-based vaccines requires the presence of antitoxin antibodies at time of toxin exposure. This is supported by the observation that despite the occurrence of many adult cases of diphtheria during a large outbreak in the former Soviet Union, a single vaccine dose raised strong antibody responses to this relatively poor immunogen. This confirmed that most patients had been immunized in childhood and had lost vaccine antibodies over time, but had persistent immune memory.[88] This immune memory was however not sufficient to protect against diphtheria, a disease characterized by a short incubation period (1–5 days). The same requirement for protective antibodies at time of exposure is frequently applied to protection against tetanus. However, tetanus does not seem to occur in previously immunized (i.e., 3 doses as adults) individuals. Whether this reflects a longer incubation time or the frequent administration of a booster dose at time of wound is unknown.

Persisting immune memory is not sufficient to protect against acute hepatitis B after the waning of vaccine-induced antibodies. When anti-HBsAg antibodies reach titers <10 IU/L, acute viral infection occurs, reflected by the appearance of anti-HBc antibodies.[89–91] However, progression to chronic liver disease has not been reported in fully immunized vaccine responders. This suggests that viral replication and re-exposure to HBsAg efficiently drives vaccine-induced memory cells into effector cells prior to the end of the viral incubation period (4–12 weeks). This process requires a sufficient number of HBsAg-specific memory B cells to be elicited, to persist and to be capable of reactivation even several decades after infant priming. Analyses of secondary responses elicited late after priming demonstrate that earlier and stronger booster responses are achieved when post-primary anti-HBsAg antibodies had reached higher titers.[77,78] This suggests the induction and long-term persistence of a higher number of memory B cells, such that protective neutralizing antibody thresholds are reached faster. Whether this confers an advantage in the race against chronic hepatitis is open to investigation. Another essential unresolved issue is whether the size of the pool of memory B cells elicited by primary immunization influences their long-term persistence, particularly in absence of antigen exposure, e.g., in low-endemicity countries. It also remains to be defined whether T cell memory responses contribute to the maintenance of vaccine-induced protection after waning of anti-HBsAg antibodies.

Glycoconjugate vaccines against encapsulated bacteria illustrate the importance of immune memory for vaccine efficacy, as well as some of its limitations. Glycoconjugate priming elicits a bona fide GC reaction, with the induction of high-affinity memory B cells that can be rapidly (4–7 days) recalled upon PS immunization.[73] Efficient priming, i.e., induction of immune memory, is readily demonstrated in children primed in infancy.[92,93] However, immune memory is also evidenced in children with Hib vaccine failure,[94] indicating that their reservoir of memory B cells failed to protect them against invasive disease. The discrepancy between the existence of memory B cells and the lack of protection may again reflect the race against microbial invasion: the time required for production of sufficient levels of circulating antibodies could be too long to interrupt bacterial invasion. Notably, secondary vaccine failures have been relatively rare, and primarily observed in countries using an early accelerated infant schedule without a booster dose,[95] the use of DTPa/Hib vaccines with lower Hib immunogenicity resulting in additional risks.[96] Thus, these priming conditions are not optimal for sustained individual protection: it is tempting to conclude that they may not elicit a sufficiently large pool of memory B cells for a sufficiently rapid interruption of bacterial invasion. Similarly, glycoconjugate vaccines against group C meningococcal disease proved much more efficacious during the first year after infant priming than during the following 3 years.[87] Thus, infant immunization fails to induce sustained protection against group C meningococcus, despite the demonstration of the induction and persistence of immune memory.[97] The requirement for boosters to confer long-term vaccine protection is also well illustrated for pertussis, where boosters are required to extend protection beyond childhood.[98] The prompt reactivation of immune memory is also not sufficient to control viral replication in the digestive tract: fecal excretion patterns were similar in subjects who were seronegative at time of oral challenge with poliovirus vaccine, whether or not they raised prompt anamnestic serum antibody responses attesting to the persistence of immune memory.[99]

Live attenuated viral vaccines (measles, rubella) are considered as the prototype inducers of life-long immunity. This derives in part from the induction of sustained antibody responses, which however tend to slowly decline in the absence of recurrent exposure[100] and might eventually result in a growing proportion of seronegative vaccinated young adults, including women of childbearing age. Whether the reactivation of immune memory will be sufficient to curtail the replication process and confer protection against measles, rubella or varicella or whether adult booster doses may become needed after microbial control are essential questions.

These questions, which are central to sustained vaccine efficacy, are usually unresolved at time of registration of a new vaccine. As an example, the relative contribution of vaccine antibodies and of immune memory to the duration of vaccine-induced protection against human papillomaviruses may currently not be predicted. Altogether, one may thus expect questions related to the nature (size, type, responsiveness) of the pool of memory cells elicited by various immunization

schedules and the relative contribution of long-term antibodies and immune memory to protection to be at the core of many vaccine studies in the next decade.

T cell vaccine responses

How do vaccines induce CD4⁺ and CD8⁺ T cell responses?

T cell vaccine responses are elicited in parallel to B cell responses (Table 2–1), through interactions with activated DCs. With the exception of PS, all vaccines induce CD4⁺ T cells, e.g., Th1 and/or Th2 cells that essentially support the differentiation of B cells (Th2) or of CD8⁺ T cells (Th1). Live vaccines also elicit CD8⁺ T cells capable of killing infected cells. The induction of both CD4⁺ and CD8⁺ T cells is essentially controlled by the nature of the initial inflammatory reaction, i.e., by vaccine adjuvants.

Vaccine antigens are taken up by immature dendritic cells (DCs) activated by the local inflammation, which provides the signals required for their migration to draining lymph nodes (Fig. 2–1). During this migration, DCs mature and their surface expression of molecules changes.[101] Simultaneously, antigens are processed into small fragments and displayed at the cell surface in the grooves of MHC (HLA in humans) molecules. As a rule, MHC class I molecules present peptides from antigens that are produced within infected cells, whereas phagocytosed antigens are displayed on MHC class II molecules.[102–104] Thus, mature DCs reaching the T cell zone of lymph nodes display MHC-peptide complexes and high levels of costimulation molecules at their surface.[105] CD4⁺ T cells recognize antigenic peptides displayed by class II MHC molecules, whereas CD8⁺ T cells bind to class I MHC-peptide complexes (Fig. 2–6).[106] Their recognition is restricted to short peptides (8–11 [CD8⁺] or 10–18 [CD4⁺] amino acids) displayed on specific MHC class I or II molecules, respectively. Antigen-specific T cell receptors may only bind to specific MHC molecules (e.g., HLA A2), which differ among individuals and populations. Consequently, T cell responses are highly variable within a population. These T cell epitopes may be generated from any region of the vaccine antigens, whether the peptide sequence is located within or at the surface of the protein. This is in contrast to B cell recognition, which is essentially limited to conformational determinants constituted by amino acids at the antigen surface. This MHC-peptide signal (signal 1) is not sufficient for T cell activation, which remain anergic or become tolerized in absence of co-stimulation (signal 2). This ensures that only naïve T cells binding to the surface of activated DCs, i.e., DCs that have sensed 'danger signals' through their Toll-like receptors and responded by a modulation of their surface or secreted molecules, receive the costimulation signals required for their activation.[105]

Activated CD4⁺ T cells essentially exert supportive functions for DCs—to which they provide signals (CD40L, etc.) resulting in further activation, for B cells (Fig. 2–2) and for CD8⁺ cytotoxic T cells (Fig. 2–6 and Table 2–8). They are elicited by each vaccine type, with the exception of unconjugated PS, and the demonstration of post-immunization CD4⁺ T cell responses does not imply a direct role in vaccine efficacy. CD4⁺ T cell activation by DCs triggers their differentiation along two distinct and mutually exclusive differentiation pathways.[105] Th1-type CD4⁺ T cells essentially produce IFN-γ and TNF-α, participating to the elimination of intracellular pathogens both directly (cytokine responses) and indirectly via their support to macrophage activation and CD8⁺ T cells differentiation (Fig. 2–6).[107] Th2-type CD4⁺ T cells essentially produce IL-4, IL-5 and IL-13 which are directly implicated in the defense against extracellular pathogens such as helminths.[108] Both Th1 and Th2 cells support B cell activation and differentiation during extrafollicular responses, whereas follicular (Tfh) CD4⁺ helper T cells provide help to GC B cells (Fig. 2–3).[36] In experimental animal models, numerous factors influence the preferential differentiation of CD4⁺ T cells towards the Th1 or Th2 pathways.[109] These determinants include the dose of antigen, lower vaccine doses being classically associated with preferential Th1 responses, and the route of administration, which targets distinct populations of DC. However, the main determinant of CD4⁺ T cell differentiation is the extent and type of DC activation by the innate system.[105] Consequently, specific adjuvants may preferentially skew CD4⁺ responses towards Th1 or Th2 responses, requiring their careful selection.

CD8⁺ T cell responses are essentially (although not exclusively, as a result of cross-presentation) elicited by vaccines that introduce antigens within the cell cytosol, ensuring their access to MHC class I molecules.[110] The induction of strong CD8⁺ T cell responses is thus currently limited to infectious, live attenuated viral or bacterial vaccines. However, novel delivery systems

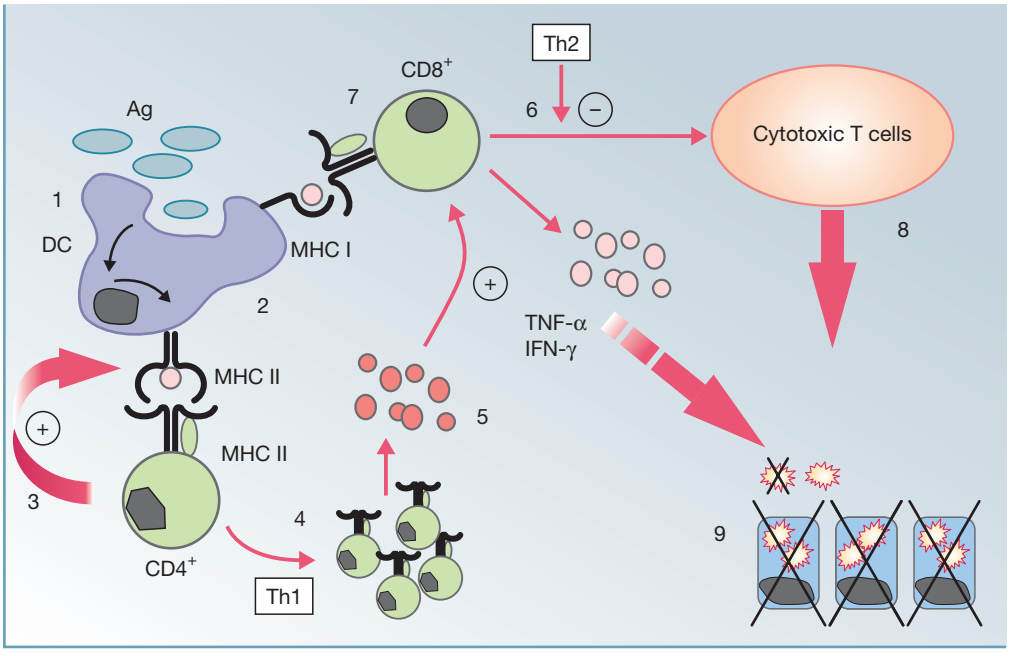

Figure 2–6 Generation of T cell effector responses. Antigens are phagocytosed by DCs (1), processed into small peptides and displayed at the cell surface in the groove of MHC class I and/or class II molecules (2). CD4⁺ T cells with the appropriate MHC-peptide specificity are activated, provide activation signals to DC (3) and differentiate in effector cells (4) that produce preferentially Th1 or Th2 cytokines. Th1 CD4⁺ T cells support (5) CD8⁺ T cell differentiation, which is in contrast inhibited (6) by Th2-like cytokines. CD8⁺ T cells recognize MHC class I-peptide complexes (7) and differentiate into cytotoxic effector cells (8) capable of killing infected cells (8) or pathogens.

Table 2–8 T cell Responses to Vaccines

Type	Mechanisms (presumed)	Function
$CD4^+$ helper T cells		
Th1	IFN-γ production	Extrafollicular B cell help
Th1	Cell contact, IFN-γ	Activation of $CD8^+$ T cells
Th1/Th2	Cell contact, CD40L	DC activation
Th2	IL-4, IL-5, IL-13	Extrafollicular B cell help
Th2	Cell contact, IL-4	Suppression of $CD8^+$ T cells
$CD4^+$ follicular helper T cells		
Th1	IFN-γ	Germinal center B cell help
Th2	IL-4, IL-5, IL-13	Germinal center B cell help
$CD4^+$ regulatory T cells		
$CD4^+CD25^+$ Treg	Cell contact, IL-2 inhibition	Suppression of $CD4^+$/$CD8^+$ IFN-γ responses.
Type 1 Treg	IL-10, TGF-β	Suppression of $CD4^+$ Th1/Th2 responses
$CD8^+$ T cells	IFN-γ, TNF-α	Killing of infected cells
Effector memory T cells	Th1/Th2 cytokines, perforin Rapid secondary effector responses in periphery	
Central memory T cells	IL-2, IL-10, CD40L	Delayed activation/proliferation in lymph nodes

Table 2–9 Determinants of Memory T cell Responses

Main factors	Determinants
Frequency of memory T cells	Magnitude of T cell expansion (initial antigen load, antigen persistence)
Phenotype of memory T cells	
Effector memory	Induction favoured by prolonged antigen persistence
Central memory	Induction favoured by rapid antigen clearance
Persistence of memory T cells	Supported by IL-15 ,IL-7

such as live-vectored vaccines or DNA vaccines are now in human trials.[111] As $CD8^+$ T cells are unique in their ability to kill cells that are chronically infected, novel vaccine targets such as HIV, HCV or malaria require their induction.

In addition to $CD4^+$ Th, follicular (Tfh) and possibly $CD8^+$ T cells, vaccine may also elicit regulatory T cells (Tregs), of which $CD4^+CD25^+$ Treg cells and type 1 regulatory T (Tr1) cells are the best characterized (Table 2–8). These regulatory T cells are elicited in an antigen-specific manner. $CD4^+CD25^+$ Treg cells potently suppress the proliferation and IFN-γ production by both $CD4^+$ and $CD8^+$ T cells, probably by direct cell-to-cell contacts and inhibition of IL-2 production.[2] Tr1 cells produce high levels of IL-10 and TGF-β, which mediate their suppressive function in both Th1- (e.g., autoimmune diseases) and Th2- (e.g., allergic responses) mediated pathologies. These regulatory T cells are induced by DCs that capture antigen in the absence of danger signals and thus remain immature during their migration to lymph nodes. In the absence of signal 2, naïve T cells do not differentiate into effector but into regulatory T cells. These Tregs play essential roles in preventing autoimmune diseases as well as allergic responses. By suppressing immune responses against self or non-self antigens, they may also limit the efficacy of vaccines when danger signals are not sufficient to elicit immunity, e.g., in chronic infections or cancer. This was recently formally demonstrated in humans by the enhancement of anti-cancer vaccine responses following Tregs depletion.[112] Numerous studies are thus currently ongoing to define the determinants of Tregs differentiation, which could lead to novel immunization strategies.

Which are the determinants of vaccine-induced T cell memory?

Effector T cell responses are short-lived, and most (>90%) effector T cells die of apoptosis within a few days. Thus, immune memory is essential to T cell vaccine efficacy. It is dependent upon three main parameters: the frequency of antigen-specific memory T cells, their phenotype and their persistence[111] (Table 2–9). Memory T cells may persist life-long even in the absence of antigen exposure.

The frequency of memory T cells reflects the magnitude of the initial T cell expansion—and that of its subsequent contraction during which few surviving cells differentiate towards memory T cells. The main determinant of the expansion phase is the amount of antigen present during priming.[113] This is a major limitation for non-replicating vaccines, which fail to reach sufficient antigen content and typically require adjuvantation and/or booster doses. The contraction phase occurs soon after antigen is cleared—which occurs faster for

non-replicating vaccines. Current efforts are thus oriented towards the optimization of the primary expansion phase through booster administration. As vaccine-induced immunity limits the subsequent take of a live vaccine by inducing its rapid neutralization, one attractive approach is the use of distinct vaccines for priming and boosting.[114–116]

The phenotype of memory T cells is also of importance. Two types of memory T cells have been identified (Table 2–8), based on their phenotype and function.[117] Effector memory cells (Tem) traffic through non lymphoid organs, where they monitor tissues for the presence of specific microbial peptides.[118] They have a high cytotoxic potential that enables them with immediate action upon pathogen recognition. In contrast, central memory T cells (Tcm) preferentially traffic through lymph nodes and bone marrow, do not exhibit much cytotoxic capacity, but have a high proliferative potential. Their role is to recognize antigens transported by activated DCs into lymph nodes and to rapidly undergo massive proliferation, generating a delayed but very large wave of effector cells.[118] Antigen persistence essentially controls the proportion of Tcm and Tem memory cells (Table 2–9): Tcm cells predominate when antigen is rapidly cleared, whereas Tem cells become preponderant when antigen persists, such as in chronic infections.[111] This is also a challenge for novel non-replicating vaccines that should induce and maintain sufficient Tem cells for immediate clearance in infected tissues. The long-term persistence of memory T cells is well established. Through homeostatic proliferation supported by specific cytokines such as IL-15 and IL-7, memory T cells may persist lifelong even in absence of antigen exposure.[119] Recent studies of the persistence of vaccinia-induced immune memory have confirmed that this applies to humans.[120–122]

How specific are vaccine immune responses?

The specificity of vaccine responses is at the center of many debates. Ideally, one would wish vaccine-induced responses to be both sufficiently broad to extend protection to non-vaccine strains (e.g., for influenza, rotavirus, *S. pneumoniae* or human papillomavirus vaccines) and sufficiently restricted not to elicit cross-reactions to allergens or self-antigens, or other undesirable non-specific effects. The specificity of vaccine responses has received added interest as a number of studies reported either positive or negative non-specific effects of vaccinations in low income countries.[123,124]

As B cells recognize conformational epitopes constituted by distant amino acids, they may bind to antigenic peptides with very distinct sequences: it has been estimated that roughly 5% of monoclonal antibodies made against 15 different kinds of viruses cross-reacted with human proteins.[125] That any viral infection is not followed by the induction or flare of an autoimmune disease highlights the importance for regulatory mechanisms to suppress responses directed against self-antigens. Indeed, the specificity of antibody responses is well controlled. Although polyclonal stimulation was suggested as capable of activating memory B cells of distinct specificities,[86] which could contribute to their homeostasis, this non-specific activation was not associated to antibody responses. Similarly, the administration of hepatitis B vaccine with CpG oligonucleotides, i.e., a potent DC activating adjuvant, did not drive pre-existing tetanus-specific B cells into antibody-producing plasma cells.[55] Vaccination with tetanus toxoid was found to expand both specific and bystander memory T-cells, but did not modulate antibody responses to unrelated antigens such that antibody production remained vaccine-specific.[126] Altogether, this indicates that the induction of cross-reactive antibody responses is extremely limited, which may be of importance to prevent undesirable reactions but limits the efficacy of vaccine-induced antibody responses to very few non-vaccine serotypes.[127]

T cells need to recognize only a few amino acids of antigenic peptides displayed by MHC molecules, which offers a much greater potential for cross-reactivity. It has been estimated that each T lymphocyte could potentially bind to millions of different peptides.[125] In addition, memory T cells readily respond to homeostatic cytokines, such that bystander memory T cells of distinct antigen-specificity may be transiently activated and expand during a flu-like illness or an immunization process.[126,128] Despite the likelihood of cross-reactive responses to infectious agents or vaccines and the relative ease with which auto-reactive lymphocytes may be elicited, vaccine-induced exacerbations of autoimmune diseases remain extremely rare, which probably reflects the efficacy of regulatory mechanisms limiting their intensity, scope and duration.[2,129]

The induction of cross-protective T cell-mediated responses has been repeatedly observed in murine experimental models, which suggested that wide spectrum viral vaccines could be based on T cell responses.[130] Convincing examples of heterologous protective immunity in humans are much more limited: neonatal BCG protects against leprosy[131] and individuals vaccinated against smallpox appear protected against monkeypox.[132] In contrast, the sharing of several T cell determinants is not sufficient for a single oral polio vaccine strain to confer cross-protection. It is thus tempting to conclude that heterologous protective immunity essentially comes at play for T-cell rather than for antibody-mediated protective responses. Accordingly, the heterosubtypic immunity conferred by live attenuated influenza vaccines[133,134] could be mediated by T cells and/or by mucosal IgA antibodies.

Non-specific effects of vaccines are occasionally associated to the fear of immune overload and subsequent enhanced vulnerability to infections, a theory which is not supported by any evidence.[135,136] Similarly, a series of observational studies linking morbidity and mortality patterns to vaccination in several low-income populations, particularly in West Africa, has generated some debate.[123,124] However, they have essentially failed to convince due to the difficulty in comparing essentially non-comparable populations, vaccinated individuals being different in many ways from those not vaccinated.

Vaccine responses at the extremes of age

The challenges of neonatal and early life immunization

According to WHO estimates, 2.5 to 3 million infants are born healthy but succumb to acute infections between the age of 1 and 12 months. These early deaths are caused by a limited number of pathogens, such that the availability of a few additional vaccines that would be immunogenic soon after birth would make a huge difference on this disease burden. Although antigen-specific B and T cell responses may already be elicited in utero, early life responses markedly differ from those elicited in mature hosts. These differences do not merely reflect the antigen naïvety of the immune system, but a true immaturity of B cells, T cells and of the microenvironment in which they differentiate.

Early life immune responses are characterized by age-dependent limitations of the magnitude of responses to all vaccines (Table 2–10). Antibody responses to most PS antigens are not elicited during the first 2 years of life, which is likely to reflect numerous factors including the slow maturation of the spleen marginal zone,[43,137] limited expression of CD21 on B cells and limited availability of the complement factors.[138] Although this may be circumvented in part by the use of glycoconjugate vaccines, even the most potent glycoconjugate vaccines elicit markedly lower primary IgG responses in young infants.[139]

Early life antibody responses are directly determined by both the prenatal (e.g., gestational age[140]) and the post-natal age at time of immunization.[138] Accelerated infant vaccine schedules in which 3 vaccine doses are given at a 1 month interval (2, 3, 4 or

Table 2–10 Limitations of Vaccine Responses at the Extremes of Life (Mechanisms Presumed)

In early life	
Limited magnitude of Ab responses to PS	Immaturity of marginal zone, low CD21 expression on B cells, limited availability of complement
Limited magnitude of Ab responses to proteins	Limited GC responses (? delayed FDC development). Inhibitory influence of maternal antibodies
Short persistence of Ab responses to proteins	Limited establishment of BM plasma cell pool (? survival niches ?)
Shorter duration of immune memory (?)	Limited GC responses (? magnitude of initial memory B cell pool)
Limited IFN-γ responses	Suboptimal APC/T cell interaction (IL-12, IFN-α)
Limited CD8+ T cell responses ?	Insufficient evidence
Influence of maternal antibodies	Inhibition of B cell but not T cell responses
In aged individuals	
Limited magnitude of Ab responses to PS	Low reservoir of IgM^+ memory B cells. Weaker differentiation into PC
Limited magnitude of Ab responses to proteins	Limited GC responses: suboptimal $CD4^+$ helper responses, suboptimal B cell activation, ? limited FDC network development). Changes in B/T cell repertoire
Limited quality (affinity, isotype) of antibodies	Limited GC responses Changes in B/T cell repertoire
Short persistence of Ab responses to proteins	Limited PC survival ?
Limited induction of $CD4^+$/$CD8^+$ responses	Decline in naïve T cell reservoir (accumulation of effector memory and $CD8^+$ T cell clones)
Limited persistence of $CD4^+$ responses	Limited induction of new effector memory T cells (IL-2, IL-7)

3, 4, 5 months) result into lower responses than schedules in which more time elapses between doses (2, 4, 6 months), or between the priming and boosting dose (3, 5, 12 months). However, the magnitude of infant antibody responses to multiple dose schedules reflects both the time interval between doses, with longer intervals eliciting stronger responses, and the age at which the last vaccine dose is administered. That postnatal immune maturation is required for stronger antibody responses is thus best demonstrated by comparing antibody responses to single dose vaccines given to antigen-naïve infants of various ages.[20,141] These studies may be confounded by the persistence of maternal antibodies, which negatively influence infant antibody responses in epitope-specific and titer-specific dependent manners.[142] Thus, multivariate analyses on a large number of infants are required to identify the main determinants of vaccine antibody responses. The induction of strong antibody responses to a single vaccine dose that would be given soon after birth unfortunately currently remains an elusive goal, and adult-like responses may eventually be only elicited in older infants.

Factors that limit the magnitude of early life antibody responses are difficult to study in human infants. Studies in which human infant vaccines were administered at various stages of the postnatal maturation of infant mice indicated that the same limitations of antibody responses affect early life human and murine responses.[138] These neonatal immunization models demonstrated that limitations of antibody responses in early life result from the limited and delayed induction of GC in which Ag-specific B cells proliferate and differentiate. This was shown to essentially reflect the delayed development of FDCs required to nucleate and support GC reactions.[143] This would explain the stepwise increase of antibody responses elicited in older infants, although direct evidence is difficult to collect and thus still limited[137] in human infants.

Importantly, neonatal immune immaturity allows the induction of immune memory, and neonatal priming may have been used to initiate vaccine responses against hepatitis B or poliomyelitis. Whether neonatal priming would similarly enhance responses to subsequent infant doses of pertussis or pneumococcal vaccines is currently under study. Although immune priming may be elicited at birth, memory responses elicited in early life could nevertheless quantitatively differ from those elicited later. This would indeed be expected: the limited magnitude of GC reactions, reflected by lower antibody responses elicited in the first year of life, is likely to be associated to the induction of a lower number of memory B cells. Whether this affects the persistence of immune memory has important implications, especially for infant immunization programs such as hepatitis B that are intended to protect throughout adult life. The duration of such responses (e.g., the boostability of hepatitis B vaccine antibody responses primed in infancy) extends for at least one decade. Whether it persists throughout a second decade is likely to be the focus of numerous studies in the next future.

Antibody responses elicited before 12 months of age rapidly wane and antibody titers soon return close to baseline levels,[97,144] which may be associated with a resurgence of vulnerability to infection.[87] This short duration of infant responses reflects the limited survival of antigen-specific plasma cells. This hypothesis was recently confirmed in infant mice,[143] in which early life bone-marrow stromal cells fail to provide sufficient survival signals to plasma cells reaching bone-marrow niches.[145] Whether this similarly limits the induction of long-lived plasma cells in human infants is unknown, but short-lived antibody responses are a hallmark of early life immunization with most—although not all (e.g., hepatitis B)—vaccines.

Isotype switching and somatic hypermutation, i.e., the affinity maturation of vaccine induced B cells, are already functional in the first year of life,[75,146–148] including in preterm infants.[140] Few studies have yet compared the affinity maturation process of vaccine responses in infants and adults, which seems to be similar (our own unpublished observations). However, several months are required for affinity maturation of vaccine antibody responses even in adults,[55] such that high-affinity responses are not observed in very young infants.

Neonatal and infant T cell responses may also differ from those elicited later in life, in particular in the induction of lower IFN-γ responses.[138] As examples, IFN-γ responses to oral polio vaccine are significantly lower in infants than in adults,[149] hepatitis B vaccine induces lower primary IFN-γ responses and higher secondary Th2 responses in early life than adults[150] and tetanus-specific IFN-γ $CD4^+$ T cell responses progressively increase with age.[151] However, vaccines are not equal in their capacity to elicit IFN-γ T cell responses in infants, and adult-like neonatal responses are notoriously elicited by BCG.[152] A limited capacity of neonatal DC to respond to various Toll-like receptor ligands by IL-12 and IFN-α production[153,154] suggests that adult-like $CD4^+$ Th1 responses are only elicited by vaccine formulations (i.e., adjuvants or delivery systems) capable of inducing a sufficiently strong DC activation to circumvent the neonatal limitations. Whether neonatal $CD4^+$ T cells have higher intrinsic requirement for antigen-specific activation and how immune immaturity affects human neonatal $CD8^+$ T cell vaccine responses requires further investigations. Such studies will be especially important for the development of novel T-cell based vaccines.

Importantly, the induction of early life B and T cell vaccine responses takes place in an environment that may be influenced by the presence of antibodies of maternal origin. IgG antibodies are actively transferred through the placenta, via the FcRn receptor, from the maternal to the fetal circulation.[155] Upon immunization, maternal antibodies bind to their specific epitopes at the antigen surface, competing with infant B cells and thus limiting B cell activation, proliferation and differentiation. The inhibitory influence of maternal antibodies on infant B cell responses affects all vaccine types, although its influence is more marked for live attenuated viral vaccines that may be neutralized by even minute amounts of passive antibodies.[156] This inhibition is epitope-specific, such that infant responses to non-immunodominant maternal epitopes may still be elicited.[157] Consequently, maternal antibodies to carrier proteins (i.e., to tetanus toxoid) mediate a specific inhibitory influence on infant responses to TT, but not to the HIB polysaccharide moiety.[158,159]

The inhibitory influence of maternal antibodies is antibody titer dependent, or rather reflects the ratio of maternal antibodies to vaccine antigen.[55] This was elegantly demonstrated in a study where Israeli infants were immunized with hepatitis A vaccine at 2, 4 and 6 months and bled immediately before each vaccine dose and at 7 months of age.[160] The first vaccine dose only induced detectable infant responses in those immunized in the absence of detectable maternal antibodies. The second vaccine dose induced detectable infant responses in those primed in the presence of maternal antibodies <1999 mIU/mL, and the third dose in those primed in the presence of maternal antibodies <3999 mIU/mL. Infant responses were only elicited when maternal antibodies reached a threshold of 300–400 mIU/mL.[160] The maternal antibody titer at which infant responses may be elicited can only be defined experimentally by comparing antibody responses in infants stratified according to maternal antibody titers at time of priming.

The extent and duration of the inhibitory influence of maternal antibodies therefore increase with gestational age,[140] e.g., with the amount of transferred immunoglobulins, and declines with post-natal age, as maternal antibodies wane.[55] Increasing the dose of vaccine antigen may also be sufficient to circumvent the inhibitory influence of maternal antibodies, as illustrated for hepatitis A[161] or measles[162] vaccines.

Although maternal antibodies interfere with the induction of infant antibody responses, they may allow a certain degree of priming, i.e., of induction of memory B cells. This likely reflects the fact that limited amounts of unmasked vaccine antigens may be sufficient for priming of memory B cells but not for full-blown GC activation, although direct evidence is lacking. Importantly, however, antibodies of maternal origin do not exert their inhibitory influence on infant T cell responses, which remain largely unaffected or even enhanced.[19,163,164] This is best explained by the fate of maternal antibodies-vaccine antigen complexes: immune complexes are taken up by macrophages and dendritic cells, dissociate into their acidic phagolysosome compartment and are processed into small peptides. These peptides are displayed at the surface of antigen-presenting cells, thus available for binding by $CD4^+$ and $CD8^+$ T cells.

Thus, the challenges for a further improvement of early life immunization strategies are to identify vaccine formulations and strategies capable of inducing after 1–2 early doses the strong primary antibody responses required for defense against certain early life pathogens. To elicit prolonged vaccine efficacy, such formulations/strategies will have to overcome the inhibitory influence of maternal antibodies for sufficient priming to occur, and to elicit more long-lived plasma cells despite the limitations of the early life bone marrow compartment. T-cell based infant vaccines will have to meet the challenge of bypassing the factors that limit the induction of Th1 early life responses. Importantly, these immunological objectives should be reached by formulations/strategies demonstrated as safe in immunologically immature hosts, adding to the challenges.

Age-associated changes in vaccine responses

Innate and adaptative antibody and T cell-mediated cellular immune responses decline with age, which increases the frequency and severity of infections and reduces the protective effects of vaccinations. Aging affects the magnitude and the persistence of antibody responses to protein vaccines,[165,166] as reflected by lower serum antibodies to influenza,[167,168] tetanus or TBE vaccines.[169] It also affects responses to pneumococcal PS vaccines, although differences in methodological issues have yielded contradictory results.[170] In contrast to infants whose antibody responses are quantitatively limited but appear qualitatively similar as those of mature individuals, limitations of antibody responses in the elderly are also associated to qualitative changes that affect antibody specificity, isotype and affinity (Table 2–10).[171,172]

The age-associated limitations of antibody responses result from the influence of a large number of underlying events.[166,173] Responses to T-independent PS vaccines are directly conditioned by a decline in the reservoir of IgM^+ memory B cells that are present at reduced numbers, differentiate less efficiently into antibody producing cells and thus limit the IgM responses to PS of aged individuals.[174] Antibody responses relying on the induction of germinal centers are also limited in senior subjects. This limitation of GCs limits B cell proliferation and differentiation, limiting the magnitude of antibody responses. It also restricts hypersomatic mutations in Ig genes, such that antibodies are of weaker affinities/functional capacities than those generated in younger individuals.[172] Last, limitations of GCs prevent efficient Ig class switching, resulting into age-associated differences in IgG1 and IgG2 subclass antibodies, e.g., to pneumococcal PS.[175] Numerous factors contribute to limit the induction of GCs in elderly persons, including factors that are intrinsic to B cells[176] and which affect other cell types. As an example, studies in aged mice have convincingly demonstrated the existence of age-related changes in FDCs, whose molecular interactions with B cells are critical for the induction and maintenance of GCs.[177,178] The limited ability of aged subjects to generate high affinity antibody responses also reflects changes in their antibody repertoire, as a result of differences in both B and $CD4^+$ T cell response capacity.[178,179]

Age-associated changes in T cell responses are reflected by a progressive decline in naïve T cells, reflecting declining thymic output. This is associated to a marked accumulation of large $CD8^+$ clones presumably resulting from prior infections. These

large T cell clones, e.g., elicited in response to cytomegalovirus (CMV) have reached a state of replicative senescence and homeostatic mechanisms negatively influence the size of the naïve and effector memory T cell subsets.[166] In response to influenza immunization healthy elderly mount $CD4^+$ responses initially similar to those of young adults, but which fail to maintain or expand such that T cell responses assessed 3 months after immunization are markedly lower than in younger adults.[180] This does not reflect a functional impairment of $CD4^+$ T memory cells[181] but a shift of the T cell pool from naïve to memory effector $CD4^+$ T cells. The failure to maintain $CD4^+$ responses reflects a lower induction of new effector memory T cells, in relation to lower IL-7 levels.[180,181] Other studies indicated that frail elderly subjects mount blunted and delayed Th1 responses to influenza vaccination, which correlated positively with their reduced total and IgG1 Ab response.[182] Limitations also affect the expansion of infection driven influenza-specific $CD8^+$ T cells.[182] Strategies to enhance vaccine-induced protection in aging individuals thus include the development of vaccine formulations capable of a stronger induction of specific B and T cell responses, for example through the selection of specific adjuvants. Nevertheless, changes in the repertoire may prove difficult to circumvent and limitations of effector memory responses and of GC responses may continue to require the more frequent administration of certain vaccine boosters (e.g., against tetanus or TBE[182]) to compensate for the brevity of B and T cell vaccine-induced responses in elderly individuals.

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